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## REVIEW

# The Greatwall kinase: a new pathway in the control of the cell cycle

T Lorca and A Castro

New data have recently established that protein phosphorylation during mitosis is the result of a controlled balance between kinase and phosphatase activities and that, as for mitotic kinases, phosphatases are also regulated during cell division. This regulation is at least in part induced by the activation of the Greatwall (Gwl) kinase at mitotic entry. Activated Gwl phosphorylates its substrates cAMP-regulated phospho protein 19 (Arpp19) and  $\alpha$ -endosulfine (ENSA), promoting their binding to and the inhibition of PP2A. Interestingly, besides the role of the Gwl-Arpp19/ENSA in the control of mitotic division, new data in yeast support the involvement of this pathway in mRNA stabilization during G<sub>0</sub> program initiation, although in this case the phosphatase PP2A appears not to be implicated. Finally, Gwl activity has been shown to be required for DNA checkpoint recovery. These new findings support the view that Gwl, Arpp19 and ENSA could function as the core of a new signaling pathway that, by targeting different final substrates, could participate in a variety of physiological functions.

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**Keywords:** Greatwall; PP2A; cyclin B–Cdc2; mitosis; mRNA; checkpoint

## INTRODUCTION

Most of the sequential events of cell division depend on protein phosphorylation. These events are controlled by a network of kinases and phosphatases that coordinate protein phosphorylation and dephosphorylation. The regulation of protein kinases during cell cycle has been largely reported, whereas the control of phosphatases has lagged far behind that of the kinases. The identification of the Greatwall (Gwl) kinase has importantly contributed to the understanding of the mechanisms controlling phosphatase activity during cell cycle.

The *Drosophila* gene coding for the Gwl kinase was first identified in 2004 in *Drosophila*. In this model Gwl was first proposed to have a role in the control of mitotic progression,<sup>1</sup> a function that was confirmed in subsequent studies in the same model.<sup>2</sup> However, the most important advance in the characterization of the signaling pathway of the Gwl kinase was performed in *Xenopus* egg extracts. Biochemical studies performed in this model allowed the identification of the targets of Gwl involved in the control of mitotic division,<sup>3–6</sup> a mechanism that was subsequently shown to be conserved in mammalian cells.<sup>7–9</sup> Finally, besides its function in the regulation of mitosis, two additional roles of Gwl have recently been identified: one of them in the DNA-damage checkpoint recovery described in the *Xenopus* egg extract model,<sup>10</sup> and the other, characterized in yeast, in the stabilization of specific mRNA transcripts required for the establishment of the G<sub>0</sub> program after nutrient deprivation.<sup>11</sup> These diverse functions of Gwl suggest an involvement of this kinase in a conserved signaling pathway that would target different downstream substrates to control a diversity of biological processes.

In this review, we discuss in detail the recent results derived from genetic, biochemical and cellular approaches showing a role of Gwl in the control of mitotic division. Moreover, we overview recent evidences about new functions attributed to the Gwl

kinase, including a role in the DNA-damage checkpoint and in the post-transcriptional mRNA protection during the initiation of the yeast quiescence program.

## ROLE OF THE Gwl KINASE IN THE REGULATION OF MITOTIC DIVISION

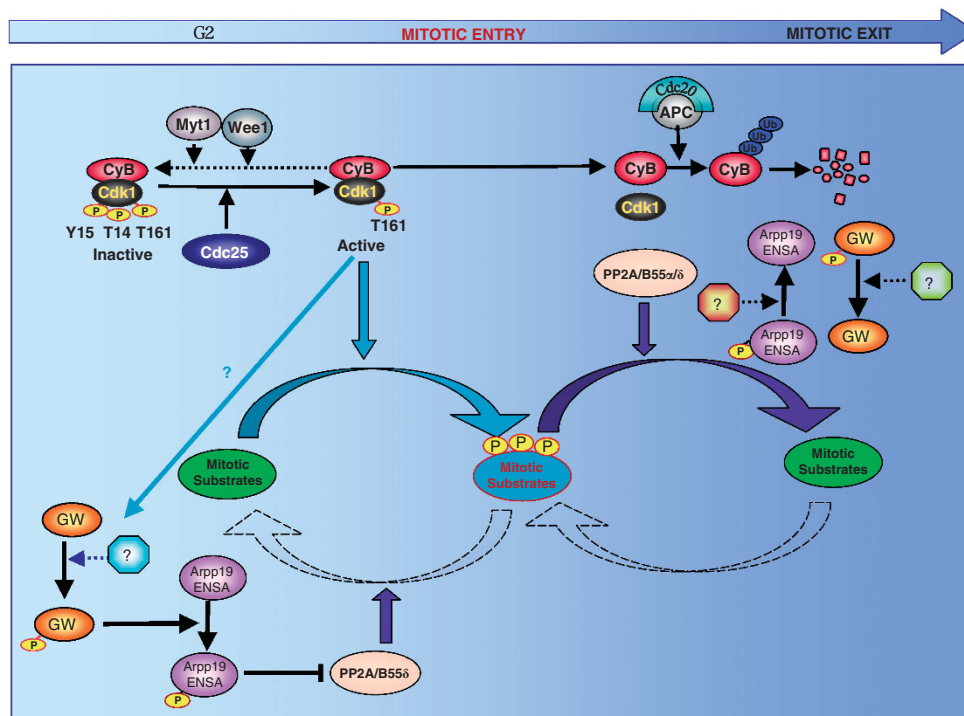
A role of Gwl in the regulation of mitotic division was first suggested in *Drosophila*.<sup>1</sup> Mutation of this protein in flies promoted improper chromosome condensation and a delay in either G<sub>2</sub> or the metaphase of larval neuroblasts. Despite the presence of undercondensed chromosomes in mutant cells, they were positive for phosphohistone H3 and for the condensing component Barren. Furthermore, 20% of cells never reached a stable metaphase and were arrested in prometaphase with an activated spindle assembly checkpoint. It was then hypothesized that Gwl was directly involved either in the chromosome condensation or in the basic cell cycle machinery. Data from a subsequent study supported the second hypothesis. Depletion of Gwl from mitotic *Xenopus* egg extracts resulted in a rapid exit of mitosis. This exit was concomitant with the inactivation of the Cdk1–cyclin B complex by the phosphorylation of the inhibitory residue Tyr 15 of Cdk1. Moreover, depletion of Gwl from cycling extracts prevented Cdk1–cyclin B activation and mitotic entry, indicating that this kinase could be required to trigger and to maintain Cdk1–cyclin B activity probably by regulating the MPF amplification loop.<sup>3</sup> Accordingly, the addition of activated Gwl to cycling *Xenopus* egg extracts promoted Cdc25 partial phosphorylation even in the absence of Cdk1, Plx1 or MAPK activity, suggesting that Gwl would be involved in the phosphorylation and activation of Cdc25. However, Gwl does not directly phosphorylate Cdc25, raising the question of the identity of the kinase responsible for this phosphorylation.<sup>12</sup>

This interpretation was significantly changed by the results showing that when the inactivation of Cdk1–Cyclin B was prevented in mitotic egg extracts by the immunodepletion of the inhibitory kinases Wee1 and Myt1, the subsequent immunodepletion of Gwl from these extracts still promoted mitotic exit.<sup>4</sup> Strikingly, this exit of mitosis was accompanied by a dephosphorylation of most of the substrates of Cdk1–cyclin B despite the high activity of this kinase. However, this massive dephosphorylation was not observed when the mitotic egg extracts were submitted to a codepletion of Gwl and PP2A. These were the first data suggesting a role of Gwl in the inhibition of PP2A, the phosphatase that would be responsible for Cdk1–cyclin B substrate dephosphorylation.<sup>4,13</sup>

The fact that PP2A was involved in Cdk1–cyclin B substrate dephosphorylation was also supported by an additional biochemical study in which the activity of PP2A was measured and compared in interphase and mitotic *Xenopus* egg extracts. In this study, the activity of PP2A was first analysed and shown to be high in interphase egg extracts and low in mitotic extracts. PP2A phosphatase is a complex of a catalytic C-subunit, a non-catalytic scaffolding A subunit and a third variable B-subunit that acts as a substrate specifier. These B subunits have been classified into four types: B (B55), B' (B56), B'' (PR72) and B''' (PR93), and some of these B subunits are coded by several related genes, resulting in different isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  for B55, and  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  for B56).<sup>14</sup> By an immunodepletion strategy in which the different B-regulatory subunits were removed, the authors identified the holoenzyme PP2A–B55 $\delta$  as the PP2A subcomplex required for Cdk1–cyclin B substrate dephosphorylation.<sup>15</sup> These results were

confirmed in an independent study in which immunodepletion of PP2A–B55 $\delta$  subcomplex corrected the inability of Gwl-depleted *Xenopus* egg extracts to enter into mitosis. These data suggested a model in which Gwl, by negatively regulating PP2A–B55 $\delta$ , confers the correct oscillatory activity of this phosphatase during mitotic division in *Xenopus* egg extracts. PP2A–B55 $\delta$  activity must be low at mitotic entry to permit Cdk1–cyclin B substrate phosphorylation, and high at mitotic exit to promote substrate dephosphorylation. The activation of Gwl at the G<sub>2</sub>–M transition ensures the inhibition of PP2A–B55 $\delta$  and thus the phosphorylation of Cdk1–cyclin B substrates, whereas the inactivation of this kinase at mitotic exit results in the reactivation of PP2A–B55 $\delta$  and in the subsequent massive dephosphorylation of these substrates (Figure 1).<sup>16</sup>

This model has been validated in human cells. Data from two different studies showed that in HeLa cells the complete knockdown of MASTL, the human orthologue of Gwl, arrested these cells in G<sub>2</sub>. Partial knockdown of MASTL enabled mitotic entry, although Cdk1–cyclin B substrates were rapidly dephosphorylated, leading to mitotic exit despite the complete absence of chromosome congression. Premature Cdk1–cyclin B substrate dephosphorylation resulted in severe cytokinesis defects, such as the formation of DNA bridges or of multinucleate cells.<sup>7,9</sup> These phenotypes are mediated by PP2A as they were rescued by either a double MASTL/PP2A knockdown or the inhibition of this phosphatase with OA.<sup>7</sup> Along the same line, PP2A–B55 $\alpha$  has been shown to be essential to allow mitotic exit in human cells.<sup>17</sup> Moreover, MASTL inactivation is required to promote mitotic exit and Cdk1–cyclin B substrate dephosphorylation in



**Figure 1.** Model of the regulatory network of Gwl during mitotic entry and exit. Results obtained in *Drosophila*, *Xenopus* egg extracts and mammalian cells about the role of the Gwl–Arpp19/ENSA–PP2A pathway are summarized in this model. At G<sub>2</sub>–M transition Gwl and Cdk1–cyclin B are activated. Cdk1–cyclin B activation promotes phosphorylation of mitotic substrates, but these phosphorylations are not maintained unless Gwl is active. The kinase responsible for Gwl activation is not identified, although the involvement of Cdk1–cyclin B in this process has been suggested. Gwl activation induces the phosphorylation of its substrates Arpp19/ENSA, which, once phosphorylated, bind and inhibit PP2A–B55 $\delta$ . This inhibition results in the complete phosphorylation of the mitotic substrates by Cdk1–cyclin B and promotes mitotic entry. At mitotic exit, cyclin B is ubiquitinated by the APC–Cdc20 ubiquitin ligase and degraded. If Cdk1–cyclin B participates in the maintenance of Gwl activity, cyclin B degradation will result in the inactivation of this kinase, the dephosphorylation of Arpp19/ENSA and the activation of PP2A–B55 $\alpha/\delta$  that will then completely dephosphorylate Cdk1–cyclin B substrates.

Cdc20 null primary mouse embryonic fibroblasts, in this case through the activation of the PP2A-B55 $\alpha$  and  $\delta$  subcomplexes.<sup>8</sup> Thus, as for *Xenopus* Gwl, in human and mouse cells MASTL is required to promote mitotic entry and/or exit through the inhibition/activation of PP2A.

Interestingly, a negative regulation of PP2A by Gwl has recently been reported in *Drosophila* oocytes during the meiotic cell cycle.<sup>18</sup> Data from a genetic screen for genes interacting with *polo* and *gwl* during syncytial embryo development identified the gene *twins* and *mts* that, respectively, encode for the orthologues of the human B55 subunit and for the catalytic subunit of PP2A. Overexpression of Gwl in eggs from *tw*s hypomorphic mutants was almost completely lethal, whereas overexpression of a kinase-dead form of Gwl had no effect on embryonic viability. Most of these eggs were arrested in the metaphase of meiosis I, suggesting a role for Gwl in promoting meiotic arrest by antagonizing PP2A-Tws. Thus, as for the mitotic cell cycle, Gwl-dependent regulation of PP2A-B55 would be required for the correct meiotic cell division.

### IDENTIFICATION OF THE SUBSTRATES OF Gwl CONTROLLING MITOTIC DIVISION

As described above, the main mitotic role of Gwl is the inhibition of the phosphatase PP2A-B55 $\delta$  that permits the maintenance of the phosphates on mitotic substrates of Cdk1-cyclin B. This massive phosphorylation is essential to promote the various events of mitosis. To advance the understanding of the mechanisms regulating this balance, it was essential to determine the mechanisms by which Gwl promoted PP2A inhibition.

The first and the simplest hypothesis was that Gwl directly modified PP2A activity by phosphorylating one of its three different subunits, a fact that was supported by the discovery that Gwl is in complex with PP2A.<sup>4,19</sup>

However, despite numerous attempts from multiple laboratories no evidence was found for Gwl to directly phosphorylate either of the different subunits of PP2A. Thus, the next most likely hypothesis was that Gwl indirectly inhibited PP2A at mitotic entry by phosphorylating an intermediate protein.

Two Gwl substrates were simultaneously identified in two different studies.<sup>5,6</sup> After biochemical fractionation of *Xenopus* egg extracts and *in vitro* phosphorylation with an ectopic Gwl kinase, these two studies identified the cAMP-regulated phospho protein 19 (Arpp19) as a substrate of Gwl. Arpp19 is very closely related to  $\alpha$ -endosulfine (ENSA), a small protein whose mutation in *Drosophila* oocytes prevents M-phase progression.<sup>20</sup> Both works reported that ectopic Arpp19 and ENSA are phosphorylated *in vitro* by Gwl on a unique serine residue (S62 and S67 of human Arpp19 and human ENSA, respectively), which promotes the binding of Arpp19 and ENSA to PP2A-B55 $\delta$  and the inhibition of this phosphatase. The addition of either Arpp19 or ENSA to interphase *Xenopus* egg extracts promoted rapid mitotic entry only if it was first phosphorylated *in vitro* by Gwl, whereas non-phosphorylated proteins or the Arpp19 S62A or the ENSA S67A mutants had no effect. Moreover, the addition of thiophosphorylated Arpp19 or ENSA to Gwl-depleted CSF extracts prevented mitotic exit, indicating that Arpp19 and ENSA are phosphorylated by Gwl and act downstream of this kinase to promote mitotic entry. However, both studies differ in the relative importance of endogenous Arpp19 and ENSA in the control of mitotic division. In this regard, in one of these works immunodepletion of ENSA did not affect mitotic entry, whereas Arpp19 depletion completely prevented entry into mitosis.<sup>5</sup> On the contrary, in the other work immunodepletion of endogenous ENSA prevented inactivation of PP2A-B55 $\delta$  and phosphorylation of mitotic substrates despite the presence of active Cdk1-cyclin B.<sup>6</sup> Further studies are required to clarify this discrepancy and to determine whether both two proteins participate in the control of

mitotic division or whether they could be involved in the regulation of the Cdk/cyclin-phosphatase balance in other phases of the cell cycle.

The fact that Gwl promotes PP2A inhibition by phosphorylating Arpp19/ENSA substrates at mitotic entry has also been supported by genetic studies in the *Drosophila* model. In a recent study, a genetic screen was performed to identify suppressors of the embryonic lethality induced by the gain-of-function Gwl mutant *Scant* when heterozygous with one mutant copy of *polo*.<sup>21</sup> Mutants of the gene *endos* encoding for the sole member of the ENSA/Arpp19 family in *Drosophila* suppressed this phenotype. Conversely, the mutation of *twins* and *mts*, the genes encoding for the regulatory B55 and catalytic C subunits of PP2A, led to complete sterility when heterozygous with *polo*<sup>1</sup>*gwl*<sup>Scant</sup>/++ females. These results suggested that Gwl and *endos* work together, whereas *endos* and *tw*s/*mt*s have opposite roles. Further analysis of the *endos* mutant revealed that it presented a similar phenotype to the *gwl* mutant, displaying an increased mitotic index and cells with low condensed chromosomes and H3-positive staining.<sup>1,21</sup> Moreover, data showed that *endos* is phosphorylated *in vitro* by *Drosophila* Gwl and that its knockdown phenotype was suppressed by co-depletion of the B55 subunit of PP2A. All these results confirmed the data obtained in *Xenopus* egg extracts showing that Gwl promotes mitotic entry by phosphorylating and activating the PP2A inhibitors Arpp19 and ENSA (Figure 1).

### MECHANISMS INVOLVED IN Gwl ACTIVATION AT MITOTIC ENTRY

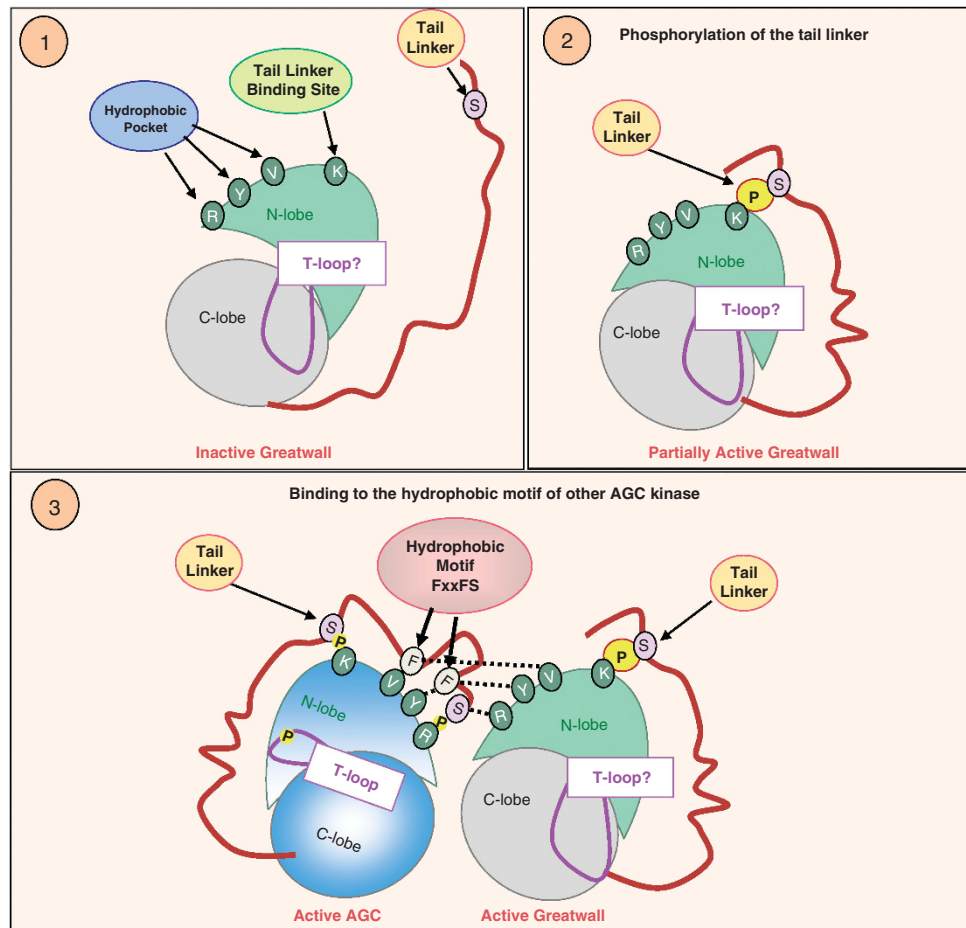
By sequence homology Gwl is classified as a member of the AGC family of kinases.<sup>1</sup> Human Gwl or MASTL displays about 60% sequence homology in their kinase domain to human PKC and PKB and 80% to human PKA. However, unlike the other AGC kinases, Gwl contains a very long insert of about 500 amino acids between kinase subdomains VII and VIII that is unique to this protein. The N-terminal and C-terminal kinase subdomains of Gwl are highly conserved in eukaryotes (94 and 84% homology in the N-terminus and 89 and 77% homology in the C-terminus of the human with *Xenopus* and *Drosophila* Gwl), whereas the central insert is less conserved (54 and 32% between these two species in the insertions). The fact that it is the sole known kinase containing a large insertion into the kinase domain makes difficult the study of the molecular mechanisms of activation and regulation. In this regard, very little data are available about the regulation of this kinase.<sup>3,22</sup>

The localization of this long insert between kinase subdomains VII and VIII suggests that it could correspond to a T-loop; however, its length does not match that of the conventional T-loop of about 20–60 amino acids. Moreover, recent data show that the point mutation or deletion of all the phospho-residues in this insert does not perturb kinase functionality, suggesting that it does not correspond to a conventional T-loop.<sup>22</sup>

Apart from this insert, most residues conserved in the AGC kinases are also present in Gwl. Activation of most AGC kinases involves the initial phosphorylation of the T-loop followed by the interaction of the C-terminal tail of the protein with its N-terminus (Figure 2). This interaction is mediated by two phospho-residues at the C-terminus, one named the tail/linker site and the other present at the hydrophobic motif (consensus sequence FxxF(S/T)(Y/F)). These two residues when phosphorylated bind at the N-terminus to the tail-linker phosphate-binding site and the hydrophobic pocket, respectively, resulting in the stabilization of the kinase-active form.<sup>23</sup>

Results from a recent study performed in the *Xenopus* egg extract model show that the hydrophobic pocket and the tail/linker phosphate-binding site are conserved and functional in Gwl and that the tail/linker site is present and phosphorylated in the





**Figure 2.** Mechanisms of activation of the Gwl kinase. Activation of Gwl involves two different steps. In the first step, Gwl is phosphorylated at its C-terminus in the Tail/Linker site (2). This phosphorylation results in the binding of the Tail/Linker site with the Tail/Linker binding site at the N-terminus of Gwl, promoting the partial activation of this kinase. However, complete Gwl activation requires the association of a phosphorylated hydrophobic motif of another unknown AGC kinase with the hydrophobic pocket of Gwl (3).

active kinase. However, surprisingly, the data also indicate that Gwl is devoid of a hydrophobic motif that is essential for activation of the majority of the AGC kinases.<sup>22</sup> Data from the measurements of the kinase activity of a significant number of Gwl mutants prompted the authors of this study to propose a model in which the first step in Gwl activation at mitotic entry would engage the phosphorylation of the tail/linker residue (Figure 2). This phosphorylation would promote the binding of the phosphate of this residue with the tail/linker-binding site at the N-terminus of Gwl, stabilizing the kinase in a partially active form. The second step would involve the association of the hydrophobic pocket of Gwl to the phosphorylated hydrophobic motif of another AGC kinase resulting in the complete activation of Gwl.<sup>22</sup> The identity of the AGC kinase involved in Gwl activation is unknown. On the contrary, there are some keys about the identity of the kinases responsible for the phosphorylation of the tail/linker site. Despite the fact that the tail/linker site does not correspond to a consensus phosphorylation motif for either Cdk1–cyclin B or Plk1, it has been shown that both kinases can *in vitro* phosphorylate this site and partially activate Gwl.<sup>22</sup> However, although Cdk1–cyclin B could be responsible for the activation of Gwl at mitotic entry,<sup>3,16,19</sup> Cdk1–cyclin B activation itself also relies on Gwl activation,<sup>3,4</sup> suggesting that another kinase would trigger Gwl and Cdk1–cyclin B activation at G<sub>2</sub>–M. Moreover, as for Cdk1–cyclin B, Plk1 appears not to be the kinase responsible for Gwl activation, as the inhibition or the knockdown of Plk1

promotes prometaphase arrest in human cells but does not prevent mitotic entry.<sup>24,25</sup> Thus, to further elucidate the pathway promoting Gwl activation at G<sub>2</sub>–M it becomes essential to identify the two unknown kinases involved in this process: the first one responsible for the phosphorylation of the tail/linker site and the second AGC kinase whose phosphorylated hydrophobic motif will bind to the hydrophobic pocket of Gwl, stabilizing its active form. Finally, to completely elucidate the regulation of Gwl activation it will be essential to determinate the phosphatase/s that are responsible for the dephosphorylation of these sites and thus for the inactivation of the kinase at mitotic exit.

### Gwl AND THE DNA DAMAGE CHECKPOINT

A role of Gwl in the DNA-damage checkpoint has been recently suggested in *Xenopus* egg extracts. In these extracts DNA-damage checkpoint activation and recovery were recapitulated by the addition and subsequent removal of biotin-labelled double-stranded oligonucleotides. Addition of these oligonucleotides promotes the activation of the DNA-damage checkpoint and phosphorylation of proteins such as Smc1 and Chk1, whereas 90 min after the subsequent removal of these oligonucleotides phosphorylation disappears as a result of the checkpoint recovery. It has been reported that extracts depleted of Gwl after double-stranded oligonucleotide addition show an increase in the phosphorylation of Smc1 and Chk1,<sup>26</sup> whereas the addition of

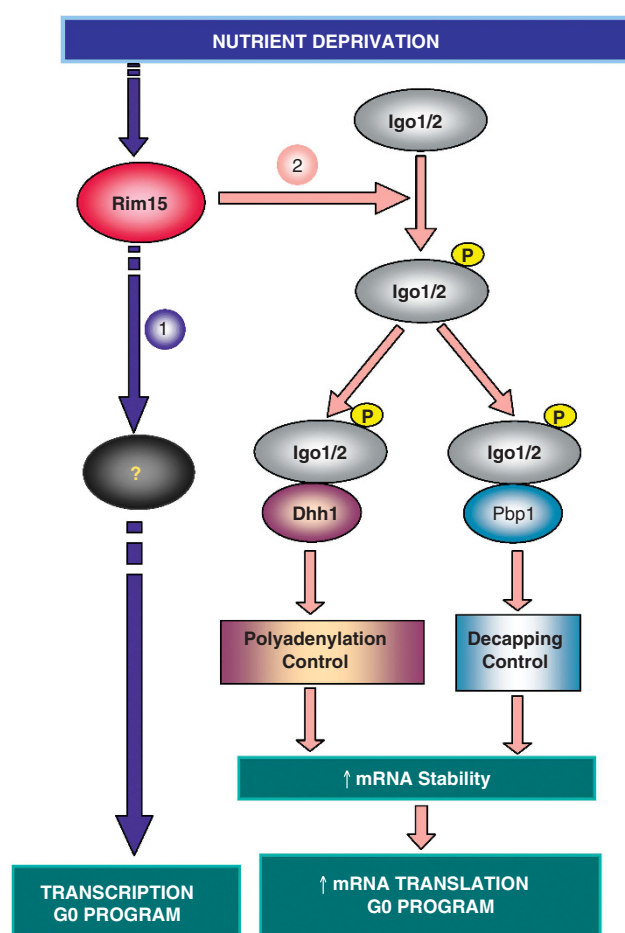
ectopic Gwl accelerates dephosphorylation of Chk1 and Smc1 and DNA-damage response recovery after oligonucleotide removal.<sup>10</sup> Moreover, Plk1 depletion appears to prevent Gwl activation during DNA-damage recovery and depletion of Gwl prevents reactivation of Plk1 during this process, suggesting that both kinases require each other to be reactivated during DNA-damage checkpoint recovery.<sup>10</sup> Finally, data supporting the binding of Plk1 to Gwl and the phosphorylation of the N-terminus of Gwl by the former kinase during DNA-damage checkpoint recovery have been reported.<sup>10</sup> All these results suggest a role of Gwl in the DNA-damage checkpoint activation and recovery; however, the exact mechanism by which Plk1 and Gwl interact and participate in this checkpoint is unclear. No direct targets of Gwl in this pathway have been reported and the effect of Gwl depletion/overexpression in DNA checkpoint activation recovery could also be explained by an indirect effect of Gwl through PP2A inhibition or Cdk1–cyclin B activation. Further studies are required to clearly establish the contribution of Gwl to the regulation of the DNA-damage checkpoint signalling.

### ROLE OF THE Gwl PATHWAY IN THE REGULATION OF mRNA STABILITY

The Gwl-ENSA/Arpp19 pathway appears to be also conserved in budding yeast. Rim15, the yeast Gwl kinase, phosphorylates Igo1/Igo2, the two orthologues of human ENSA and Arpp19 at the site equivalent to the *Xenopus* ENSA and Arpp19.<sup>11</sup> Moreover, Rim15 *in vitro* phosphorylates the equivalent site in human ENSA and Arpp19 proteins. In yeast, Rim15 is essential to promote G<sub>0</sub> program initiation after nutrient deprivation by controlling the expression of specific genes. Inhibiting the kinase TORC1 by rapamycin activates Rim15 and drives cells into the quiescent-like state. Rapamycin treatment results in a 2.8-fold increase in the expression of genes implicated in G<sub>0</sub> arrest in wild-type but not double Igo1/Igo2 mutant cells. This increase appears to be the result of a dual role of the Rim15 kinase (Figure 3). The first one, of which the mechanism is unknown so far, would involve a direct activation of gene transcription. The second one is mediated by the phosphorylation of the Igo1/2 proteins and results in the stabilization of specific mRNAs by preventing mRNA degradation via 5'–3' mRNA decay pathway. Accordingly, Igo1/2 bind Pbp1 and Dhh1, two proteins implicated in the regulation of mRNA stability by controlling mRNA polyadenylation and mRNA decapping, respectively (Figure 3). Pbp1/Dhh1–Igo1/2 interaction is increased when Rim15 is activated by the addition of rapamycin and decreased in the absence of Rim15 and when the interaction is analysed by using an Igo1 in which the serine of the Rim15 phosphorylation site is mutated to alanine. Intriguingly, in the *Drosophila endos<sup>00003</sup>* mutant, Polo kinase expression is markedly reduced in ovaries although *polo* mRNA expression appears to be unaffected.<sup>20</sup> Moreover, human ENSA and Arpp19 are able to partially replace Igo1/2 function in yeast,<sup>11</sup> suggesting that a role of the Gwl-ENSA/Arpp19 pathway in the regulation of mRNA stability could be also conserved in higher eukaryotes.

### Gwl AND POLO: AGONIST OR ANTAGONIST KINASES?

Compelling data support the hypothesis of a functional interaction between the Gwl and Polo kinases during mitotic division; however, the nature and the role of this interaction appear to be contradictory in the different models. Evidence of a direct interaction of Gwl and Polo has been reported in the *Xenopus* egg extracts model. In this model, a binding of these two kinases has been shown in interphase extracts as well as in DNA-damage checkpoint-recovering extracts. Interestingly, Gwl is reactivated during DNA-damage checkpoint recovery and this reactivation is dependent on the Plx1 kinase, suggesting that Gwl could be phosphorylated and activated by Plx1.<sup>10</sup> Accordingly, *in vitro*



**Figure 3.** Role of the Gwl pathway in the regulation of mRNA stability. In budding yeast, nitrogen and/or carbon limitation causes activation of Rim15, the yeast orthologue of Gwl. This activation promotes two different effects. (1) The stimulation by an unknown pathway of the transcription of specific genes required for G<sub>0</sub> program initiation (violet arrows) and (2) the phosphorylation of the Arpp19/ENSA orthologues Igo1/Igo2 (pink arrows). The latter event stimulates the association of these proteins with Dhh1 and Pbp1, and promotes the stabilization of specific mRNAs of the G<sub>0</sub> program through the regulation of mRNA polyadenylation and decapping.

phosphorylation studies indicate that Plx1 phosphorylates a serine residue in the C-terminus of Gwl that has an essential role in kinase activation.<sup>22</sup> Thus, in this model, Gwl and Polo appear to function synergically to promote mitotic entry.

On the contrary, an antagonistic genetic interaction between these two kinases has been reported in *Drosophila* syncytial embryo.<sup>18,21,27</sup> The gain of function allele of *Drosophila* Gwl, *Scant*, produces embryos that have greatly reduced viability only when heterozygous with one mutant copy of *polo*.<sup>27,28</sup> In these embryos, centrosomes tend to detach from the nuclear envelope during migration in the syncytial divisions, leading to aberrations in the subsequent mitosis. Increasing the *gwl<sup>scant</sup>/polo* ratio by duplication of *polo*<sup>+</sup> suppresses this phenotype,<sup>27</sup> whereas reducing this ratio by using a Polo inhibitor enhances it.<sup>18</sup> Moreover, revertant alleles of *Scant* that restore fertility to *gwl<sup>scant</sup>/polo* heterozygous females are recessive alleles deficiencies of *gwl*.<sup>27</sup> These data suggest an antagonistic relationship between these two kinases. A model has been provided to explain these apparent antagonistic functions of Gwl and Polo in *Drosophila*.<sup>21</sup> It is known that Polo interacts with phosphorylated partners at mitotic entry and progression, but with dephosphorylated partners at mitotic exit.

At anaphase, Polo would bind at least one dephosphorylated protein and this interaction would be required for centrosome–nuclear envelope binding. The gain-of-function mutant of Gwl, *Scant*, would transiently decrease PP2A activity, resulting in increased phosphorylation of the Polo-partner. This would reduce Polo-partner interaction and would finally result in the detachment of centrosome from the nuclear envelope. This phenotype would be enhanced in the heterozygous *polo<sup>1</sup>gwl<sup>scant</sup>/++*, in which the levels of Polo kinase are decreased. However, the Polo partner and the mechanisms by which this kinase maintains centrosome attachment are completely unknown so far.

## Gwl AND HUMAN DISEASES

Despite the fact that the Gwl/MASTL kinase has an essential role in the regulation of cell division and probably due to the fact that data on this topic only emerged in the last 3 years, little is known about the implication of this protein in human diseases. The involvement of MASTL in human pathology has only been demonstrated for the autosomal dominant inherited thrombocytopenia. This disorder is characterized by moderate thrombocytopenia and incomplete differentiation of megakaryocytes. The gene for the autosomal dominant thrombocytopenia was first mapped to a region on human chromosome 10p11–12.<sup>29</sup> This corresponded to a missense mutation that segregated perfectly with this disorder and that caused a substitution of a cytosine to a guanidine at nucleotide position 565 of the FLJ14813 gene. This gene was subsequently shown to encode for the MASTL kinase and the reported mutation caused a predicted substitution of aspartic acid for glutamic acid (E167D), a mutation that appears not to affect kinase activity (our unpublished results). Moreover, the knockdown of this protein in zebrafish promotes a reduction of circulating thrombocytes, suggesting that MASTL could be involved in the production of platelets although the exact physiological mechanism is unknown so far.<sup>30</sup>

In addition to the role of MASTL in the thrombocytopenia disease, some lines of evidence suggest that PP2A, ENSA or Arpp19 could also be implicated in pathologies such as cancer or Parkinson diseases. In this line, PP2A has been reported to function as a tumour suppressor and the deregulation of the activity of this phosphatase participates in the tumoral process.<sup>31</sup> Moreover  $\alpha$ -Synuclein, the major fibrillar protein component of the Lewy bodies present in diseases such as Parkinson, Alzheimer and Down syndrome,<sup>32</sup> associates with ENSA and Arpp19, and regulates in this way its physiological activity.<sup>33</sup> However, a deeper analysis is required to establish the involvement of the MASTL–Arpp19–ENSA pathway in these pathologies.

## CONCLUSIONS

It is becoming increasingly clear that in addition to the regulation of Cdk1–cyclin B activity the control of phosphatases promoting dephosphorylation of mitotic substrates has an essential role in the control of mitotic entry and exit. The understanding of the mechanisms regulating phosphatases has only just begun with the identification of the Gwl/Arpp19–ENSA/PP2A–B55 $\delta$  pathway. However, a great number of questions are still unanswered. Which is/are the kinase/s involved in Gwl activation at mitotic entry? Which is/are the phosphatase/s required to promote Gwl or Arpp19/ENSA inactivation at mitotic exit? What is the contribution of Arpp19 and ENSA to mitotic division and to other phases of the cell cycle? Are there other PP2A subcomplexes in addition to PP2A–B55 $\alpha/\delta$  or other phosphatases that are regulated by Gwl? How are Arpp19 and ENSA regulated during the cell cycle? We are just at the beginning of the understanding of these regulatory pathways that control mitosis. Moreover, it is likely that this balance between kinases and phosphatases is not exclusive to the mitotic division, but probably represents a more general

mechanism for controlling protein phosphorylation in other phases of the cell cycle and other physiological processes. Thus, we are just likely visualizing the tip of a very large iceberg whose complete comprehension will allow us not only to understand the mechanisms regulating mitosis but also to have a more complete global view of other biological process.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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